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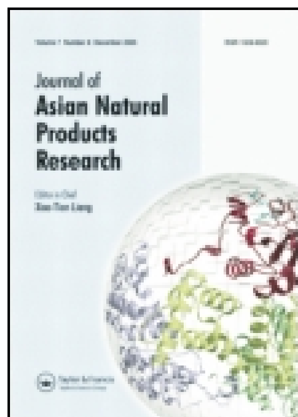

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Yi Li [a b] , Jun Luo [a] , Qiang Wang [a] & Ling-Yi Kong [a]

[a] School of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing, 210009, China

[b] Testing and Analysis Center, Nanjing Normal University, Nanjing, 210046, China

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Two new limonoids from the stem barks of *Chukrasia tabularis* var. *velutina*

Yi Li[ab], Jun Luo[a], Qiang Wang[a]* and Ling-Yi Kong[a]*

[a]*School of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing 210009, China;* [b]*Testing and Analysis Center, Nanjing Normal University, Nanjing 210046, China*

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Two new phragmalin-type limonoid orthoesters, C-15 enolic acyl type and 13/14/18-cyclopropanyl type, named chukvelutilide H (**1**) and tabularin R (**2**), together with three known limonoid orthoesters (**3**–**5**), were isolated from the stem barks of *Chukrasia tabularis* var. *velutina*. The structures of these two new compounds were elucidated on their extensive HR-ESI-MS, 1D, and 2D spectroscopic analysis including HSQC, HMBC, and ROESY experiments.

Keywords: *Chukrasia tabularis* var. *velutina*; phragmalin-type limonoid; orthoester

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1. Introduction

The stem barks of *Chukrasia* genus plants, *Chukrasia tabularis* and its variety *C. tabularis* var. *velutina*, have been used traditionally as astringent, antidiarrheal, and anti-influenza agents in China [1,2]. Phytochemical researches of these two plants revealed that phragmalin-type limonoids were the major components [3–16], and fatty acids, steroids, and flavones also existed [17,18]. In our previous research, a series of phragmalin-type and 16-norphragmalin-type limonoids were isolated from the chloroform fraction of its ethanol extraction [11–16]. Further investigation led to the isolation of five limonoid orthoesters (Figure 1), one new C-15 enolic acyl phragmalin-type chukvelutilide H (**1**), and four 13/14/18-cyclopropanyl phragmalin-type limonoids (**2**–**5**), including a new one tabularin R (**2**) from the stem barks of *C. tabularis* var. *velutina*. The structures of these two new compounds were elucidated on their extensive HR-ESI-MS, 1D, and 2D spectroscopic analysis including HSQC, HMBC, and ROESY experiments. Herein, the isolation and structural elucidation of these novel compounds were reported.

2. Results and discussion

Chukvelutilide H (**1**) was isolated as white amorphous powder with the molecular formula $C_{44}H_{54}O_{20}$ as determined by the HR-ESI-MS at *m/z* 925.3086 $[M + Na]^+$. The ^1H and ^{13}C NMR spectral data of **1** (Table 1) and the data from decouplings and the subsequent 2D NMR studies (HMBC, HSQC, and NOESY), especially the signals of two overlapped protons at δ_H 1.92 showing HMBC correlations with Me-28 (δ_C 14.2), C-1 (δ_C 84.5), and C-4 (δ_C 45.7), and characteristic β-substituted furanyl ring [δ_H 6.40, 7.28, and 7.59; δ_C 122.2, 109.8, 142.6, and 141.1], suggested that **1** was a phragmalin-type limonoid

*Corresponding authors. Email: cpu_lykong@126.com; qwang1949@126.com

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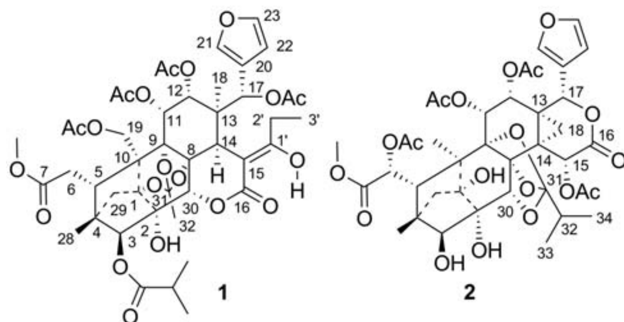

Figure 1. Structures of new compounds.

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[11–15,18] with four acetoxyls, one isobutyryl, and a methoxyl. A HMBC correlation (Figure 2) between a quaternary carbon at δ_C 119.8 (C-31) and a single methyl signal at δ_H 1.64 (H-32) indicated that **1** was an orthoester derivative [19].

The presence of a characteristic enolic proton signal at δ_H 13.72, and the β-ketolactone carbon signals at δ_C 180.0 (C-1′), 92.1 (C-15), and 169.9 (C-16) indicated that **1** was a C-15 enolic acyl phragmalin-type limonoid derivative [3,11], which was confirmed by the HMBC correlations (Figure 2) from the enolic proton signal at δ_H 13.72 to two of β-ketolactone carbon signals (C-1′ and C-15), and H-14 (δ_H 3.34) to three carbon signals of β-ketolactone. In the HMBC

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** in $CDCl_3$.

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1		84.5	23	7.28, br s	142.6
2		77.0	28	0.98, s, 3H	14.2
3	4.90, s	83.0	29a	1.92, s, 2H	39.6
4		45.7	29b		
5	3.20[a]	37.2	30	5.46, s	73.8
6a	3.20[a]	32.2	31		119.8
6b	2.40[a]		32	1.64, s, 3H	20.9
7		172.8	1′		180.0
8		80.5	2′	2.40, 2.57, m, 2H[a]	25.7
9		82.7	3′	1.26, t (7.5), 3H	11.1
10		47.5	OH-1′	13.72, s	
11	6.44, d (2.5)	69.3	OCH_3-7	3.71, s, 3H	51.9
12	4.56, d (2.5)	70.4	$OCOCH(CH3)_2$-3		176.7
13		44.6		2.90, m	34.2
14	3.34, s	43.8		1.34, d (7.0), 3H	19.0
15		92.1		1.32, d (7.0), 3H	19.0
16		169.9	OAc-11		168.9
17	5.80, s	70.2		2.12, s, 3H	20.8
18	1.49, s, 3H	18.0	OAc-12		168.8
19a	4.55, d (11.5)	66.0		1.58, s, 3H	19.7
19b	4.27, d (11.5)		OAc-17		168.5
20		122.2		1.97, s, 3H	20.7
21	7.59, br s	141.1	OAc-19		171.0
22	6.40, br s	109.8		2.07, s, 3H	21.0

Note: [a]Signal pattern unclear due to overlapping.

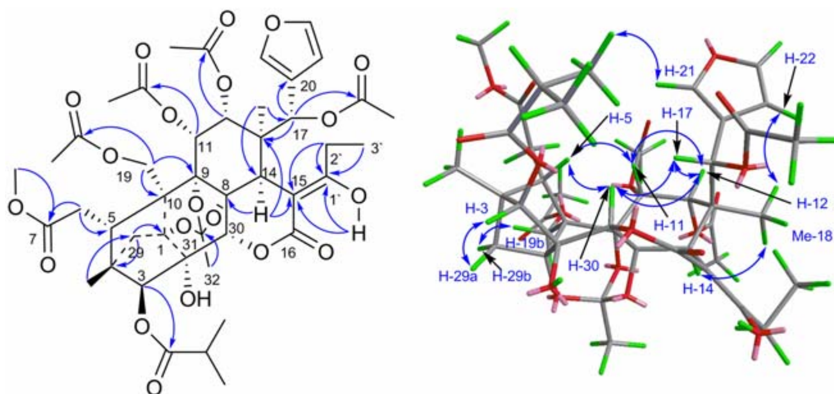

Figure 2. Key HMBC (→) and NOE (←→) correlations of **1**.

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spectrum of **1**, the correlation from a methyl signal of ethyl at δ_H 1.26 (3H, t, $J = 7.5$ Hz, H-3′) to the carbon signal at δ_C 180.0 (C-1′) suggested that a biosynthetically extended propionyl group was attached at C-15. The aforementioned evidence and the similarity of the ^1H and ^{13}C NMR spectral data between **1** and chukvelutilide A indicated that these two natural products possessed the same C-15 enolic propionyl phragmalin skeleton with a 1,8,9-*ortho*-acetate and an C-16/C-30 δ-lactone ring, which was determined by single-crystal X-ray diffraction experiment [11]. Furthermore, the isobutyryl and four acetyls were assigned at OH-3, OH-11, OH-12, OH-17, and OH-19, respectively, according to their corresponding HMBC correlations. NOESY correlations (Figure 2) from H-5 to H-30 and H-11, from H-17 to H-12 and H-30, and from H-30 to H-12 indicated a β-orientation of these five proton signals in **1**. NOESY correlations of H-14 with Me-18, H-29a with H-3, and H-29b with H-19b revealed that these protons adopted α-orientation. NOESY correlation between the methyl signal of isobutyryl-3 and H-21 of the furan ring also confirmed the α-orientation of H-3. Thus, the relative configuration of **1** was established by a NOESY experiment as depicted (Figure 2), which was the same as chukvelutilide A obtained by the X-ray crystallographic study [11]. Thus, the structure of **1** was established as shown in Figure 1.

Tabularin R (**2**), white amorphous powder, has a molecular formula $C_{39}H_{46}O_{19}$ as established by its HR-ESI-MS at *m/z* 841.2520 $[M + Na]^+$, indicating 17 degrees of unsaturation. The whole features of its ^1H and ^{13}C NMR spectra (Table 2) indicated that compound **2** was also a phragmalin-type limonoid, especially the signals of a β-substituted furanyl ring [δ_H 6.49 (br s), 7.36 (br s), and 7.52 (br s); δ_C 122.3, 109.9, 143.0, and 142.0] and typical H-29 proton signals of 4,29,1-ring-bridge [δ_H 1.67 and 2.12 (d, 11.5); δ_C 40.2] in phragmalins. HMBC correlations from the proton signals of an isopropyl [δ_H 2.16, (m), 1H; δ_H 1.07 (d, $J = 7.5$ Hz), 3H; δ_H 1.05 (d, $J = 7.5$ Hz) 3H] to a typical orthoester carbon signal at δ_C 118.9 suggested that the orthoester moiety in **2** was an isobutylate group [6,15]. In HMBC and HSQC spectra of **2**, two proton signals [δ_H 2.64, dd (7.0, 2.0) and δ_H 1.45, d (7.0)] of a methylene (δ_C 15.8, C-18) showed HMBC correlations with C-8 (δ_C 87.0), C-12 (δ_C 66.7), C-13 (δ_C 29.0), C-14 (δ_C 25.3), C-15 (δ_C 70.7), and C-17 (δ_C 71.2), which revealed that C-13, C-14, and methyl-18 formed a cyclopropanyl ring in **2**. The aforementioned information suggested that **2** was a phragmalin-type

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **2** in $CDCl_3$.

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1		84.5	21	7.52, br s	142.0
2		77.2	22	6.49, br s	109.9
3	3.81, s	85.8	23	7.36, br s	143.0
4		44.0	28	1.09, s, 3H	17.1
5	2.93, br s	43.3	29a	2.12, d (11.5)	40.2
6	6.23, br s	71.6	29b	1.67, d (11.5)	
7		171.8	30	4.25, s	79.6
8		87.0	31		118.9
9		84.4	32	2.16, m[a]	29.2
10		49.8	33	1.07, d (7.5), 3H	15.5
11	5.67, d (4.0)	67.2	34	1.05, d (7.5), 3H	16.9
12	5.48, d (4.0)	66.7	OCH_3-7	3.80, s, 3H	53.2
13		29.0	OAc-6		171.1
14		25.3		2.26, s, 3H	21.1
15	7.31, d (2.0)	70.7	OAc-11		169.3
16		166.4		2.03, s, 3H	21.0
17	6.38, s	71.2	OAc-12		169.9
18a	2.64, dd (7.0, 2.0)	15.8		1.52, s, 3H	19.2
18b	1.45, d (7.0)		OAc-15		168.9
19	1.35, s, 3H	17.6		2.21, s, 3H	21.0
20		122.3			

Note: [a]Signal pattern unclear due to overlapping.

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limonoid orthoisobutylate with 13/14/18-cyclopropanyl ring [6,15]. HMBC correlations between proton signals of skeleton and ester carbonyl carbons indicated that C-6, C-11, C-12, and C-15 were acetoxylated. The location of the orthoester moiety and lactone ring could not be determined directly by the HMBC spectrum since no valuable correlations from proton signals of skeleton to orthoester carbons C-31 (δ_C 118.9) and C-16 (δ_C 166.4) were observed. On the basis of chemical shifts about key locational carbons, compound **2** possessed 8,9,30-orthoester group and C-16/C-17 δ-lactone ring as tabularin C [6], which was determined by single crystal X-ray diffraction experiment. The ROESY correlations from H-17 to H-30, H-11, and H-12, from H-30 to H-5, and from H-11 to H-5 and H-12 indicated that these protons adopted a β-orientation [5]. Correlations from Me-19 to H-29a and from H-3 to H-29b revealed that these protons were α-orientated, which was also consistent with tabularin C [6]. Thus, the structure of **2** was established as shown in Figure 1.

Compounds **3**–**5** were identified to be known compounds tabularins A (**3**) [6], J (**4**) [9], and E (**5**) [8], by comparing their NMR and MS spectral data with the published values.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a JASCO P-1020 polarimeter. IR (KBr discs) spectra were recorded by a Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Bruker ACF-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz). Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and a Mariner ESITOF spectrometer (HR-ESI-MS), respectively. All solvents used were of analytical grade (Jiangsu Hanbang Sci. & Tech. Co. Ltd., Huaian, China). Silica gel (Qingdao Haiyang Chemical Co. Ltd.,

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Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and RP-C_{18} (40–63 μm, FuJi, Aichi, Japan) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C_{18} column (20 × 200 mm) and 1100 Series Multiple Wavelength detector.

3.2 Plant material

The air-dried stem barks of *C. tabularis* var. *velutina* were collected from Xishuangbanna, Yunnan Province, China, and were authenticated by Professor Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (NO. 2006-MML) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3 Extraction and isolation

The air-dried stem barks (10 kg) were extracted by refluxing 95% ethanol three times. The EtOH extract was concentrated under reduced pressure (2000 g) and then extracted with $CHCl_3$ to give the chloroform extract (300 g). The oily chloroform extract was dissolved in 2 L of 50% MeOH and H_2O and then extracted with petroleum ether (PE). After removal of the fatty components, 210 g of extraction was obtained, which was subjected to a silica gel column eluted with $CHCl_3$/MeOH in gradient (1:0 to 1:2) to afford eight fractions (Fr. A–H) according to TLC profiles. Fr. B (20 g) was chromatographed on a column of silica gel, eluted successively with a gradient of PE–EtOAc (3:1 to 1:2), to give four sub-fractions (Fr. B1–B4), and compound **3** (100 mg). Fr. B4 (2.1 g) was chromatographed on a column of silica gel, eluted successively with a gradient of PE–EtOAc (3:1 to 1:2) to give **5** (15 mg). Fr. C (22 g) was chromatographed on a column of silica gel, eluted successively with a gradient of PE–EtOAc (4:1 to 1:2) to give eight sub-fractions (Fr. C1–C8). Fr. C3 (1.6 g) was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH/H_2O (1:1 to 3:1) to give four sub-fractions (Fr. C3a–C3d), then Fr. C3b (20 mg) was separated by preparative HPLC using CH_3CN/H_2O (70:30, 10 ml/min) as the mobile phase to give **1** (3 mg). Fr. C6 (3.0 g) was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH/H_2O (1:1 to 3:1) to give five sub-fractions (Fr. C6a–C6e), then Fr. C6d (50 mg) was separated by preparative HPLC using CH_3CN/H_2O (55:45, 10 ml/min) as the mobile phase to give **4** (10 mg). Fr. D (30 g) was chromatographed on a column of silica gel eluted successively with a gradient of PE–EtOAc (5:2 to 1:2) to give seven sub-fractions (Fr. D1–D7). Fr. D3 (8.0 g) was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H_2O (5:5 to 7:3) to give four sub-fractions (Fr. D3a–D3d), then Fr. D3d (100 mg) was purified by preparative HPLC using CH_3CN-H_2O (45:55, 10 ml/min) as the mobile phase to give **2** (6 mg).

3.3.1 *Chukvelutilide H (**1**)*

White amorphous powder; $[\alpha]_D^{25}$ −48 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3457, 2976, 1738, 1640, 1606, 1369, 1219 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; negative ESI-MS *m/z*: 901.6 $[M - H]^-$ (100); positive ESIMS *m/z*: 920.5 $[M + NH_4]^+$ (100); HR-ESI-MS *m/z*: 925.3086 $[M + Na]^+$ (calcd for $C_{44}H_{54}O_{20}Na$, 925.3101).

3.3.2 *Tabularin R (**2**)*

White amorphous powder; $[\alpha]_D^{25}$ −25 (*c* 0.07, CH_3OH); IR (KBr) ν_{max} 3432, 2958, 1753, 1712, 1641, 1431, 1372, 1247, 1228 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 2; positive ESI-MS *m/z*: 819.2 $[M + NH_4]^+$ (100); HR-ESI-MS

m/*z*: 841.2520 $[M + Na]^+$ (calculated for $C_{39}H_{46}O_{19}Na$, 841.2526).

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